Supplementary Materials and Methods

Prethymic T cell development defined by the expression of Paired Immunoglobulin-like Receptors

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Mice

Balb/c mice were purchased from SLC (Shizuoka, Japan). KSN *nu/nu* mice were maintained in our animal facility.

Coculture with stromal cells

To assess the potential of single cells for their ability to give rise to B cells, cells were individually cultured on a monolayer of the stromal cell line TSt-4 (Ohmura *et al*, 1999) for 10 days, and B cell generation was determined by examining the CD19 expression. Myeloid potential was assessed similarly, but the stromal cell line used was PA6 (Nishikawa *et al*, 1988), and Mac-1 was used as the myeloid cell marker.

RT-PCR

RT-PCR was performed as described previously (Kawamoto *et al*, 2000). Primers and amplicon sizes are shown below. Cycling times and temperatures were as follows: denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec, and elongation at 72˚C for 30 sec. Amplification was performed for 25 cycles for *β-actin* and 35 cycles for others.

Table S1. Oligonucleotide Sequences

RT-PCR

Primers

Name	Sense Primer	Antisense Primer	Product Size (bp)
ikaros	5’-GAGGCATGGCCAGTAATGTT-3’	5’-AGGCCGTTCACCAGTATGAC-3’	281
PU.1	5’-AGATGCACGTCCTCGATACT-3’	5’-TTGTGCTTGGACGAGAACTG-3’	345
GATA-1	5’-TCCCAGTCCTTTCTTCTC-3’	5’-ACAATTCCCACTACTGCTGC-3’	544
GATA-2	5’-TGACCATGAAGAAGGAAGGG-3’	5’-AGACTGGAGGAAGGGTGGAT-3’	238
GATA-3	5’-TCGGCCATTCGTACATGGAA-3’	5’-GAGAGCCGTGGTGGATGGAC-3’	290
Tcf-1	5’-CCAGCTTTCTCCACTCTACG-3’	5’-TCAAGGATGGGTGGGTGAAC-3’	280
EBF	5’-AACTGGCTGTGAATGTCTCG-3’	5’-CACATGGGAGGGACAATCA-3’	450
Pax5	5’-TCCTCGGACCATCAGGACAG-3’	5'-CCTGTTGATGGAGCTGACGC-3’	394
mb-1	5’-GCCAGGGGGTCTAGAAGCCC-3’	5’-TCACTTGGCACCCAGTACAA-3’	308
λ5	5’-TTGAGGGTCAATGAAGCTCAGAAGA-3’	5’-CTTGGGCTGACCTAGGATTG-3’	337
Notch1	5’-CCCAGCAGGTGCAGCCACAG-3’	5’-GGTGATCTGGGACGGCATGG-3’	460
Hes1	5’-GCCAGTGTCAACACGACACCGG-3’	5’-TCACCTCGTTCATGCACTCG-3’	247
Deltex	5’-ATCAGTTCCGGCAAGACACA-3’	5’-TACGCGTTCTGGATGGTGAT-3’	160
Rag2	5’-CCCAGAGAACCACAGAAAAAT-3’	5’-TAACCACCCACAATAACAAAT-3’	336
EpoR	5’-CTGACTTGGCCTCAAAGC-3’	5’-GCTCTGAGTCTGGGACAAGG-3’	275
lck	5’- CATTCCCTTCAACTTCGTGG-3’	5’- TAATGGCGGACTAGATCGTG-3’	297
CD3ε	5’- ATCACTCTGGGCTTGCTGAT-3’	5’- TAGTCTGGGTTGGGAACAGG-3’	149
β-actin	5’-TCCTGTGGCATCCATGAAACT-3’	5’-GAAGCACTTGCGGTGCACGAT-3’	315

PCR analysis of TCRβ, TCRγ and IgH chain gene rearrangement

DNA equivalent to 1000 cells and 250 cells were used for detection of D-Jβ rearrangement and V-Jγ rearrangement, respectively. Primers were: Dβ1, 5'-TTATCTGGTGGTTTCTTCCAGC-3'; Dβ2, 5'-GCACCTGTGGGGAAGAAACT-3'; Jβ2.6, 5'-TGAGAGCTGTCTCCTACTATCGATT-3'. Vγ4, 5'-AGTGTTCAGAAGCCCGATGCA-3'; Vγ5, 5'-ACAACTGTGGTGGATTCCAGA-3'; Jγ1, 5'-AGAGGGAATTACTATGAGCT-3', Vγ1.1, 5'-CTTCCATATTTCTCCAACACAGC-3'; Jγ4, 5'-ACTACGAGCTTTGTCCCTTTGG-3'; DSF, 5'-AGGGATCCTTGTGAAGGGATCTACTACTGTG-3'; J_{H4}, 5'-AAAGACCTGCAGAGGCCATTCTTACC-3'. The reaction volume was 20 μl containing 5 μl of the cell extract, 1.5 μl of 10x PCR buffer, 0.16 μl of 25 mM dNTPs, 0.4 μl of each primer (10 mM), and 0.6 U of Taq polymerase. Thermocycling conditions were as follows: 5 min at 94˚C followed by 35 cycles of 1 min at 94˚C, 1 min at 60˚C for D-Jβ rearrangement or at 55˚C for V-Jγ rearrangement, 2 min at 72˚C, and 10 min at 72˚C. To detect immunoglobulin D-J_H rearrangement, 1.0μl of each primer (10mM) was added, and the following PCR conditions were used: 35cycles of 1 min at 94˚C, 1.5 min at 60˚C, and 2 min at 72˚C. The elongation time at 72˚C was prolonged 3 sec in each cycle; the final elongation step was 10 min.

Supplementary References

Kawamoto H, Ikawa T, Ohmura K, Fujimoto S, Katsura Y (2000) T cell progenitors emerge earlier than B cell progenitors in the murine fetal liver. *Immunity*, **12:** 441-450

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Ohmura K, Kawamoto H, Fujimoto S, Ozaki S, Nakao k, Katsura Y (1999) Emergence of T, B and myeloid lineage-committed as well as multipotent hematopoietic progenitors in the aoorta-gonad-mesonephros region of day 10 fetuses of the mouse. *J Immunol*, **163:** 4788-4795